REVIEW

Epigenetic Regulation of Centromere Chromatin Stability by Dietary and Environmental Factors

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ABSTRACT

The centromere is a genomic locus required for the segregation of the chromosomes during cell division. This chromosomal region together with pericentromeres has been found to be susceptible to damage, and thus the perturbation of the centromere could lead to the development of aneuploidic events. Metabolic abnormalities that underlie the generation of cancer include inflammation, oxidative stress, cell cycle deregulation, and numerous others. The micronucleus assay, an early clinical marker of cancer, has been shown to provide a reliable measure of genotoxic damage that may signal cancer initiation. In the current review, we will discuss the events that lead to micronucleus formation and centromeric and pericentromeric chromatin instability, as well transcripts emanating from these regions, which were previously thought to be inactive. Studies were selected in PubMed if they reported the effects of nutritional status (macro- and micronutrients) or environmental toxicant exposure on micronucleus frequency or any other chromosomal abnormality in humans, animals, or cell models. Mounting evidence from epidemiologic, environmental, and nutritional studies provides a novel perspective on the origination of aneuploidic events. Although substantial evidence exists describing the role that nutritional status and environmental toxicants have on the generation of micronuclei and other nuclear aberrations, limited information is available to describe the importance of macro- and micronutrients on centromeric and pericentromeric chromatin stability. Moving forward, studies that specifically address the direct link between nutritional status, excess, or deficiency and the epigenetic regulation of the centromere will provide much needed insight into the nutritional and environmental regulation of this chromosomal region and the initiation of aneuploidy. *Adv Nutr* 2017;8:889–904.

Keywords: centromere, chromatin instability, DNA methylation, centromeric transcription, nutrition, micronucleus

Introduction

Centromeres are portions of each chromosome that have fundamental roles during cell division—from duplication of the genetic material to equal segregation of the chromosomes into each new nucleus. However, due to their inherent architectural complexity, our understanding of centromeric arrangement, assembly, and control is relatively incomplete (1). The primary function of the centromere is to allow assembly of kinetochore protein complexes and stabilize the pairing of sister chromatids. The subsequent binding of microtubules, and further mobilization toward opposite poles, appears to be independent of the centromeric DNA sequence. This process strictly relies on the epigenetic control of the pericentromeric and centromeric chromatin, as well as chromatin modifiers, which together with DNA methylation and demethylation events help to establish the intricate landscape of this essential region (2).

During metaphase, the centromere is a chromosomal region that holds a primarily constricted arrangement upon which the kinetochore complex is assembled, thus safeguarding the division of sister chromatids. Chronic diseases such as obesity and cancer (initiation and progression) are accompanied by chromosomal damage (3), whereas a particular feature of cancer is the presence of visible cellular anomalies that clearly indicate the presence of abnormal ploidy within the dividing cell. Such abnormal chromosomal arrangements and segregation aberrations can be explained to a certain extent by the degree of chromatin

This collaboration is supported by a University of Illinois at Urbana-Champaign/Universidad Nacional Autónoma de México Joint Research Grant from the Agricultural, Consumer and Environmental Sciences (ACES) Office of International Programs.
Author disclosures: DH-S, RSS, PO-W, and Y-XP, no conflicts of interest.
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Abbreviations used: CCAN, constitutive centromere associated network; CENH3, centromeric histone 3; CENP, centromeric protein; CIN, chromosomal instability; Daxx, Death domain–associated protein; DNMT1, DNA methyltransferase 1; HJURP, Holliday junction recognition protein; HSATII, human satellite II; L1, long interspersed nuclear element 1; ncRNA, noncoding RNA; RNAi, interference RNA; satncRNA, satellite noncoding RNA; siRNA, small interfering RNA.

instability generated during cell division. The link between chromatin instability and these diseases has been attributed to changes within the epigenetic landscape (4), which ultimately lead to the deleterious abnormalities that characterize disease processes.

Environmental factors, including diet, exert their influence on cellular homeostasis to a great extent through epigenetic mechanisms that provide a dynamic link between nutrition and disease (4). The ability of nutrients to modify the way in which a cell adapts to the environment through diverse mechanisms, and how these processes could lead to the development or prevention of chronic diseases, is the focus of extensive research. The cytokinesis-block micronucleus assay allows us to assess genotoxicity in response to environmental stimuli by detecting affected cells that have a higher propensity of developing a micronucleus (5–7); however, the mechanisms behind the chromosomal instability (CIN) that leads to the formation of micronuclei are poorly understood. Several molecular processes have been associated with increased CIN, namely acentric chromosome fragments, dicentric chromosomes, telomere-end fusions, silencing of cell cycle checkpoint genes, centromeric DNA amplification, and epigenetic regulation of centromeric, pericentromeric, and telomeric chromatin. Altogether, these environmentally sensitive processes may affect the way chromosomes segregate during cell division, promoting the build-up of genetic lesions that could hamper the viability of the cell.

Cell division is an intricate process by which cells duplicate their genetic material and create 2 identical viable daughter cells to support cellular regeneration, making the control and supervision of this process critical for cellular and organismal survival (8). Given the conspicuous role of centromeric and pericentromeric regions for the control of chromatin stability and cellular division, the present review will first provide a comprehensive overview of what is currently known about the precise functional roles of the centromere and pericentromeric chromatin. We will then discuss how transposons and noncoding RNAs (ncRNAs) affect the condensation state of the chromatin. Finally, we will present a perspective establishing a link between centromere chromatin instability and environmental and dietary factors that likely interact to have genotoxic consequences.

Functions, Structure, and Regulation of Centromeric and Pericentromeric Chromatin

Chromosomes tightly pack and protect genetic information during cell division to ensure proper segregation of the code that will help to maintain a cell's integrity. Despite their different content and appearance, all chromosomes undergo uniform duplication and preparation during the cell cycle, as well as the separation of the sister chromatids and the formation of the 2 daughter cells during mitosis. During this partition, many chromatin structures are of vital importance for the correct assembly of the kinetochore at the centromere, allowing for the division machinery to pull the sister chromatids apart toward opposite poles, followed by the formation of the new nuclei, thus guaranteeing equal partitioning of genetic information (8).

Function and structure of centromeric and pericentromeric DNA

The centromere is a key chromosomal structure whose structure and the processes that regulate its establishment and functionality have remained elusive even after the conclusion of the Human Genome Project (9). The challenge for studying centromeric chromatin likely lies in its highly repetitive nature (**Figure 1**), making it virtually impossible to sequence by using currently available technologies. Centromeric DNA is arranged in 171-bp tandem repeats of alphoid DNA (α-satellites) rich in A-T dinucleotides wrapped around centromere-residing histones (10, 11). Together, these components establish the centromere chromatin assembly that will be strictly regulated and meticulously folded during cell division to drive cellular separation. Kapitonov et al. (12) provided a complete analysis of the different centromeric satellites by describing the nature of noncryptic centromeric satellites, which are different from unique genomic DNA and can be divided into classic satellites I, II, III, and IV; these can also be organized according to their size, conservation, and similarities into 5 suprachromosomal families. The complexity and high degree of structural variation highlight the forthcoming challenges for the complete sequencing of the mammalian centromere. Adding to this complexity is the observation that novel ectopic centromeres or "neocentromeres" can arise at distant genomic locations, and these do not include any of the features mentioned for traditional centromeres (13); therefore, although the centromeric DNA sequence is not specifically required for the overall kinetochore assembly (1, 14), the high degree of sequence conservation of all centromeres suggests that there might be a function associated with its sequence. As will be discussed in later sections, current research has expanded our understanding of the role of these noncoding transcripts (from constitutive centromeric and pericentric heterochromatin) in kinetochore recruitment and stabilization through their interaction with centromeric proteins (CENPs) (14); therefore, despite the fact that the centromere sequence does not participate in the spatial specification, it can regulate the stability of centromeric chromatin by generating noncoding centromeric transcripts.

Pericentromeric DNA arrangement is also important for the maintenance of centromeres and stability of the kinetochore-centromere interaction. Emil Heitz was the first to describe pericentromeres as heterochromatic regions in moss nuclei, observing that such regions forego decondensation after the conclusion of mitosis (15). Subsequently, numerous studies have found that the heterochromatic state is crucial for centromere function (16–18). Pericentromeres, like telomeric regions (19), are gene-poor regions located at both ends of the centromere chromatin. Their main structural role is to act as boundary elements to delineate each arm of the chromosome and the coding regions within. Specific DNA sequences and chromatin landscape define this region, having more diverged α-satellite repeats that contain transposable elements, such as long interspersed element 1 (L1) and short interspersed element (SINE) retrotransposons

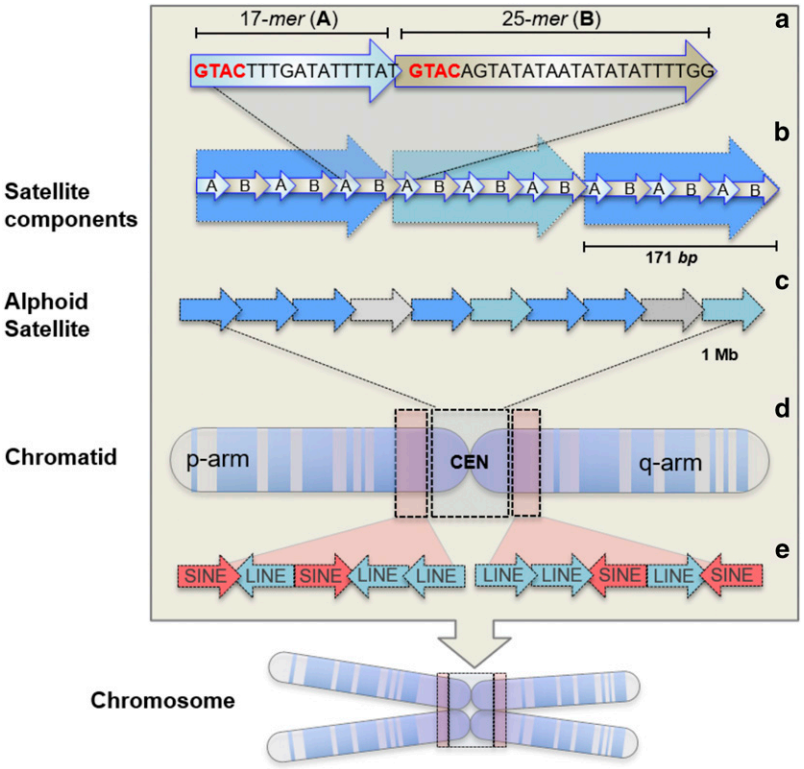

FIGURE 1 Centromeric and pericentromeric DNA. The general sequence of a centromeric DNA with a 17- or 25-mer basic unit (a) arranged in a sequential unidirectional fashion (b), which altogether describes the ~176-bp satellite repeats that contain different combinations of the basic units (c). The size and organization of the α-satellites within the centromere vary between chromosomes (d). Nevertheless, transposable elements are found within pericentromeric regions flanking all centromeres (e). CEN, Centromeric DNA; LINE, long interspersed nuclear element; p-arm, small arm of the chromosome; q-arm, large arm of the chromosome; SINE, short interspersed element.

near the edges (2) (Figure 1). L1 transposons are highly active and abundant in humans and are a source of interindividual genetic variation (20), which is due to the ability of these elements to mobilize and insert at distant genomic locations to alter the genomic sequence. Despite their evident adjacent localization on each chromosome, it is clear that the link between centromeres and pericentromeres does not rely on their individual DNA sequences, but rather on their complementary function during cell proliferation. Flanking heterochromatic regions are required for de novo kinetochore formation, as has been observed when localized heterochromatin protein 1 binding is induced, which allows for the proper kinetochore-centromere interphase assembly (21). Consequently, assembly of kinetochore proteins and their regulators is closely dependent on the stability of pericentric regions that, in turn, specify the centromere.

Previous studies considered α-satellite sequences to be crucial for the interaction between centromeric loci and kinetochore proteins, but the discovery of de novo centromeres named "neocentromeres" that are located at distant genomic loci and lack satellite sequences challenged this theory. Current research is attempting to determine the basis for the formation and persistence of satellite-deficient centromeres. Recently, it was shown that a full-length L1 retrotransposon transcript (FL-L1b) is an essential structural and functional component of the neocentromeric chromatin (22), which provides a possible mechanism for neocentromere localization and establishment at nonsatellite regions. The relation between pericentromeric elements and centromere maintenance will be highlighted in later sections.

Centromeric and pericentromeric DNA methylation

DNA methylation is a genomewide epigenetic mechanism that has proven to be a strong and heritable way to regulate the expression of many genes. Yamagata et al. (23) described centromeres and pericentromeres as genomic structures that can discriminate between the somatic and germ cell lineage by exhibiting a differential DNA methylation pattern that can accurately define the fate of the cell, where the germ lineage would display hypomethylated centric and pericentric DNA. DNA methylation at the centromere is of great importance for the establishment and maintenance of a competent chromatin landscape, because studies with maintenance methyltransferase DNA methyltransferase 1 (*Dnmt1*) showed that the loss of CpG hypomethylation in Dnmt1$^{-/-}$ embryonic stem cells produced pericentric chromatin reorganization that resembled that of the inactive X chromosome, and the lower methylation levels were accompanied by macroH2A deposition, with re-establishment after treatment with transgenic Dnmt1 (24). RNA transcripts that emanate from these regions constitute a novel function of centromeric DNA methylation, which has challenged the previously believed "silent centromeric chromatin" hypothesis, showing their ubiquitous nature in different species (*Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Mus musculus*, and *Homo sapiens*). Therefore, DNA methylation acts as an underlying permissive set of boundaries for transcription, maintaining the overall hypermethylated state and the intermittent hypomethylated pockets within the centromere. This explains the tolerance for centromeric transcription without a compromise for the heterochromatic state (25), which has

been suggested to be made possible through the positioning of pericentric nucleosomes (26). Interestingly, at many developmental stages or during carcinogenesis, pericentromeres undergo a conformational transformation from either facultative to constitutive heterochromatin, or vice versa. This switch mechanism is DNA- and H3K9 methylation–sensitive and is regulated, to a great extent, by BEN domain containing 3, a protein associated with pericentric regions (27). Therefore, as with all DNA, pericentric DNA methylation appears to control the binding of many factors that will, in turn, regulate the state of the chromatin in response to cellular and environmental cues.

CENP localization and function

Centromeric DNA is closely linked with a great variety of proteins, including histone H3 variant CENP-A, CENP-B, CENP-C, and a specific H3-CENP-T/W (8), all of which constitute a DNA-associated protein network (21). These CENPs perform a variety of functions—acting as histones, DNA-binding proteins, and scaffolding structures—thus regulating both the chromatin state and centromeric function. Westhorpe and Straight (28) provide a complete architectural view of CENP-A and other centromere-specific DNA-binding CENPs and their post-translational modifications that participate in the epigenetic control of chromosome segregation.

CENP-A as the key centromeric identifier. It is thought that the deposition of CENP-A, rather than the underlying DNA sequence, is the hallmark of all functional centromeres. Octameric CENP-A nucleosomal arrangements at human centromeres provide a looser DNA terminus and fewer base pairs (121 bp) than the conventional H3 nucleosomes (29, 30), constituting the fundamental unit of epigenetic specification of the centromere (31). Consequently, elegantly distributed arrays of CENP-A nucleosomes and their tails harness the functionality of the centromere and ensure the attachment of the necessary centromere-kinetochore intermediate protein complex (32). Thus, without CENP-A nucleosome establishment at centromeres, the structural arrangement that leads to the kinetochore assembly would not occur.

During mitosis, every component of the cell, including CENP-A, needs to be correctly positioned for the division event to proceed. The intrinsic properties of CENP-A allow it to regulate its "cell cycle–restricted assembly" and its "quantitative mitotic transmission," which depend on the CENP-A targeting domain (CATD), guiding the future position of the centromere (33). CENP-A overexpression in aggressive cancers was shown to produce their ectopic accumulation driven by the Death domain–associated protein (Daxx) histone chaperone, which was able to promote a higher tolerance toward DNA damage, prevent transcription factor CCCTC-binding factor (CTCF) binding and, in turn, remodel adjacent chromatin (34). In *Arabidopsis*, the reduced-fertility phenotype was caused by the depletion of centromeric histone 3 (CENH3) (CENP-A homolog), thus causing its deficient loading onto the centromere and consequent missegregation of chromosomes, leading to increased micronucleus formation (35). Therefore, any perturbation in CENP-A expression or loading could ultimately lead to mitotic defects that compromise cell division.

The temporal distribution of the CENPs throughout the cell cycle demands rigorous control, which is particularly true for CENP-A, which constitutes a hallmark for epigenetic maintenance of centromere positioning (33). At the first stages of the cell cycle, CENP-A histones are loaded onto the centromere, advancing to the S phase to achieve centromeric DNA duplication, which leads to the dilution of CENP-A to half of that which continues through the cell cycle. By the G2 phase, more CENP-A is synthesized and incorporated into a soluble preloading complex with histone H4 and the Holliday junction recognition protein suppressor of chromosome mis-segregation 3 ($HJURP^{Scm3}$) chaperone (36), thus facilitating its deposition at the centromere, which does not happen until the late stages of mitosis and G1 phase (37). The mechanism by which the HJURP chaperone directs the temporal localization of CENP-A was recently described by Wang et al. (38) to occur through the interaction of HJURP with the mitotic regulator Mis18β, and possibly by its recognition of histone 3 lysine 4 dimethylation (H3K4me2), as observed in human artificial chromosomes (39). Therefore, the loading of CENP-A at the centromeres is regulated by many cell cycle–specific protein complexes, which monitor the proper arrangement and spacing of CENP-A nucleosomes at the centromere. Similarly, in rice (*O. sativa*), the deposition of CENH3 (CENP-A homolog) is bordered by differentially methylated satellites of centromeric DNA, and these regions of DNA hypomethylation help to discriminate from surrounding H3 regions and establish precise CENH3 localization (40). Likewise, heterochromatic pericentromeric-specific histone modifications and their modifiers Suv39, Dicer, Chp1 [RNA-induced initiation of transcriptional silencing complex (RITS) component, described in later sections], and Swi6 (HP1α in humans) are also able to direct the deposition of CENP-A to the centromere, as seen in fission yeast (41). Therefore, it is evident that the CENP-A nucleosome establishment is the result of a concerted effort from both chromatin modifiers and pericentromeric heterochromatin.

The role of the constitutive centromere assembly network. Kinetochore assembly requires not only CENP-A but a vast variety of scaffolding proteins that will, at the appropriate time, provide a bridge between centromeric chromatin and the expanding microtubules. Several centromeric proteins have been proposed to bind to CENP-A and facilitate the assembly of the centromere. CENP-C, which is a component of the constitutive centromere assembly network (CCAN), is thought to directly bind to CENP-A and provide a stable scaffold for the recruitment of other CCAN proteins (42), which guarantees centromeric-specific recognition at the time of CCAN assembly.

Another relevant protein is CENP-B, a highly conserved protein of the mammalian CCAN (43) that binds centromeric DNA with high affinity. It was found that CENP-B

is a target of α-N-methyltransferases in humans, which enhances CENP-B binding through trimethylation, which might alter assembly, disassembly, or maintenance of the centromere (44). The functions of CENP-B extend beyond the simple scaffold forming; in *Schizosaccharomyces pombe*, a member of the CENP-B family, Abp1, has been shown to associate with H3K4 methyltransferase Set1 and recruit class I/II histone deacetylases to repress the activation of Tf2 long terminal repeat (LTR) retrotransposons, presumably by long-range chromatin organization regulation (45). Thus, CENP-B not only functions as a centromere-specific scaffold but also as a recognition epitope for important chromatin modifiers. Interestingly, the methylation state of the centromere has been found to interfere with the binding of CENP-B, which, in turn, is known to interact with alphoid sequences by recognizing the 17-bp CENP-B box (46, 47). In this case, the increased methylation state, when located at the CENP-B box, restricts the access of CENP-B to its target sequence (48), altering the consecutive CCAN protein association.

Similar to CENP-A, other proteins that contain a histone fold have been proposed to be present at centromeric regions. For instance, CENP-W is a newly identified centromeric chromatin protein that can bind nucleolar component (nucleophosmin, B23), and thus it has been proposed to bind RNA and DNA (49), which, in turn, may confer stability to the centromere. CENP-W associates with other proteins that contain histone properties like CENP-T to form a dimer, and the dimer, in turn, can bind CENP-S/X to form a heterogeneous tetramer CENP-T/W/S/X (28). However, further research needs to be conducted to detect the proposed assembly of CENP-T/W/S/X heterotetramers within centromeres. In a similar way, recent data suggest a novel role for proteins CENP-C and CENP-I of the CCAN, which work together to recruit M18BP1 of the Mis18 complex of the kinetochore and further direct the incorporation of CENP-A to the centromeric nucleosomes (50).

CENPs are vital for the specification of centromeric chromatin, but only constitute a small proportion of the kinetochore and centromere components required for the separation of chromatids. Numerous additional proteins and processes take place during kinetochore assembly and arrangement of the centromeric chromatin (51). Such processes include post-translational modifications to histone tails or chromatin remodeling events that allow additional protein complexes to direct the kinetochore apparatus to the centromere. Although not discussed in this review, it is important to note that chromatin modifiers and non–CENP-interacting proteins (KMN complex, Chromosome Passenger Complex, Spindle Assembly Checkpoint, and many others), constitute an important regulatory network that acts as a surveillance mechanism to further guide the centromere-kinetochore interaction.

Centromeric and Pericentromeric ncRNAs

As discussed previously, the interaction of centromeric and pericentromeric chromatin architectures, as well as the recruitment of chromatin remodelers, are crucial for the appropriate conformation of the centromere; however, the discovery of centromeric and pericentromeric transcripts have contributed to the understanding of the complexity of the centromere chromatin assembly (13, 52, 53).

Centromeric and pericentromeric ncRNAs can be short or long nucleotide sequences that are transcribed from specific regions that were once thought to be inactive, because they are buried within heterochromatin segments. More recently, it was shown that together with histone modifications, centromere transcriptional regulation functions as a key recruitment factor for centromere proteins and assembly of CENP-A domain, which, in turn, provides another layer of regulation to the already intricate centromere-kinetochore interaction (54). The relevance of such transcripts thought to be nonexistent and nonfunctional is that they are actually vital for different cellular processes, such as cell growth and differentiation, stress, and affect of chromatin organization (52). Therefore, the existence of conserved sequences at the centromeric region appears to defy the assumption that satellite sequences are not crucial for the stability of the centromeric chromatin.

Centromeric and pericentromeric transcript biogenesis

The transcripts emanating from centromeric regions are important in helping to strengthen the interactions that produce heterochromatic environments at centromeric regions, and thus their regulation needs to be closely supervised. To date, 3 mechanisms for heterochromatin formation have been proposed in fission yeast (*S. pombe*). First, an interference RNA (RNAi) pathway through the RNA-dependent RNA polymerase (RDRP) produces a double-stranded pericentric transcript that is further chopped by Dicer, yielding a small interfering RNA (siRNA) that can bind RITS, which, in turn, mediates the coupling with a nascent pericentric transcript capable of recruiting the CLRC (Clr4 complex) histone methyl transferase complex that is vital for heterochromatin maintenance. A second mechanism bypasses RDRP through a secondary stem loop structure. Finally, a third (but poorly understood) proposed mechanism appears to be RNAi independent, possibly comprising exosomal processing (55), which is mediated by 3′-5′exonuclease Triman in association with Argonaute, which produce mature primary microRNA and siRNAs, in turn producing de novo assembly of heterochromatin at centromeric repeats (56). However, hypermorphic transcription of centromeric retroelements results in the disruption of CENP-A localization, thus contributing to centromere chromatin instability in the tammar wallaby model (*Macropus eugenii*) (57). Therefore, the known pathways that lead to the production of functional ncRNAs are diverse, but the end result appears to be the production of transcripts that stabilize heterochromatic settings for the proper functioning of the centromere.

An association between the modifications produced by the action of the transcripts and the transcripts themselves appears to be that of a positive feedback loop that reinforces the generation of heterochromatin. As discussed in the

context of chromatin organization, different histone modifications can regulate the interaction of the histone core and the bound DNA; in the case of the centromere, modifications on CENP-A and similar centromeric-specific proteins have a profound impact on the structural organization that will determine the proper function of the centromere-kinetochore complex. The transient mono-ubiquitination of centromeric H2B is able to increase transcription of centromeric elements in *S. pombe* and human cells, a process that is cell cycle–dependent and primarily regulated by RNF20. The loss of this enzyme produces centromere inactivation and kinetochore assembly failure (58). Thus, neighboring histone modifications mediate the transcription of centromeric elements, which, in turn, promotes the consolidation of heterochromatic boundaries. Recently Liu et al. (59) were able to dissect the intricate role of centromeric transcripts on the stable localization of Sgo1, the cohesion protector. As the transcripts are emanating from the centromere by the action of Pol II, Sgo1 is recruited and thus sister chromatid cohesion is enabled.

Given their linear proximity, ribosomal DNA repeats embedded within centromeric sequences allow for their association with the nucleolus, highlighting the importance of such structures and the establishment of heterochromatin. For instance, TIP5, a component of the nucleolar-remodeling complex, is able to mediate heterochromatin formation, as well as maintain the silencing of centromere-adjacent ribosomal RNA genes and of major and minor satellite repeats (60).

The role of Dicer-mediated RNAi formation on centromeric chromatin stability

The role of RNAi in the formation and maintenance of centromere and pericentromeric chromatin has been established in several vertebrate models. Dicer and convergent antisense transcription are crucial for primary siRNA generation, because these promote heterochromatin formation by establishing the H3K9 methylation marks (H3K9me) that are normally present at centromeres (61), thus exerting a critical role in centromeric chromatin stability. In zebrafish, for example, *Dicer1* knockout and knockdown by target-selected inactivation produce developmental arrest at day 10 with a concomitant microRNA decrease, indicative of Dicer's decisive role in vertebrate development (62). Moreover, targeted replacement of the RNase III domain of Dicer1 in mice with a PGK-neo^r expression cassette generates a lethal phenotype (63). Conditional targeting of mouse embryonic stem cells through Cre-loxP technology produced a viable cell with defective differentiation accompanied by reduced DNA methylation and H3K9me3 at the pericentric regions (64). Together, this underscores the critical function of the Dicer enzyme in stem cell maintenance during early developmental stages, by promoting other epigenetic events at centromeric regions.

In a similar way, the absence of Dicer generates defects in cell division. The loss of Dicer function is linked to the increased accumulation of α-satellites from centromeric DNA in Dicer-mutant chicken-human hybrid DT40 cells that contain human chromosome 21, and the increase in centromeric transcripts was accompanied by aberrant localization of heterochromatin proteins Rad21 and BubR1 (65). Chan and Wong (54) provide a broad list of transcripts originating from these regions that can be detected in different model systems. The RNA-processing enzyme Dicer is thus an unequivocally vital component for the recruitment of several chromatin modifiers and for stimulation of epigenetic changes within centromeric regions.

Transcriptional regulation of centromeric and pericentromeric chromatin

Different environmental factors that induce physiologic stress produce DNA damage and oxidative stress can affect the transcription pattern of pericentromeric regions (66). Jolly et al. (67) discussed, for the first time to our knowledge, how environmental stress–induced heat shock transcription factor 1 (HSF1) activation leads to the formation of nuclear stress granules, structures thought to increase transcription of centromeric Satellite III (Sat-III) due to their association with RNA pol II, showing the close relation between environmental stress and centromeric repeat transcription. The transcriptional regulation of heterochromatic regions in response to physiologic stress indicates that, upon induction of cellular stress (autophagy, senescence, oxidative stress, inflammation, etc.), silent centromeric regions will be upregulated, which, in turn, can regulate protein synthesis (68). Certain compounds can induce transcription of Sat-III repeats to different extents; etoposide, aphidicolin, and methylmethane sulfonate have a low effect, followed by UV radiation type C and hyperosmotic stress; and finally, heat shock and cadmium are strong inducers of Sat-III transcription (69). Interestingly, the application of physiologic heat shock produces the accumulation of histone chaperone Daxx at centromeric and pericentromeric regions where it mediates the incorporation of H3.3, and the depletion of Daxx produces an epigenetic landscape switch by increasing active H3K4me2 at pericentromeres (70), which would lead to altered transcript expression and abnormal centromere function.

Other satellite repeat transcripts have been found to be critical for chromosomal segregation. For example, the silencing of Sat-I caused abnormal segregation and an aberrant nuclei phenotype, accompanied by increased Aurora B kinase activity and Chromosome Passenger Complex dislodgment from the centromere (71). Transcription of centromeric and pericentromeric transcripts seems to be a conserved mechanism in response to cellular stress. In *Arabidopsis*, transient induction of transcripts originating from heterochromatic loci is achieved after exposure to different temperature conditions, bypassing repressive epigenetic marks (72). Interestingly, the presence or absence of both centromeric and pericentric transcripts is indicative of epigenetic deregulations in response to cellular stress that lead to cancer (73). The dynamic nature of centromeric and pericentromeric transcripts shows the plasticity of the heterochromatin at these genomic loci; therefore, regardless

of the source of cellular stress, the impact on the stability of the centromere and its transcripts appears to be altered.

As discussed previously ("Centromeric and pericentromeric transcript biogenesis"), several advances have uncovered the many pathways in which ncRNAs participate in centromere stability; however, more recently, the emerging role of ncRNA has focused on the initiation and progression of cancer. Particularly, the expression of satellite ncRNAs (satncRNAs) that are able to enhance the carcinogenic potential (74, 75), as seen in many types of cancer such as breast, pancreatic, colon, hepatocellular, ovarian, lung, kidney, prostate, etc., indicates the importance of such transcripts during oncogenesis (76). The putative pathways that seemed to be mediated by satncRNA have been reviewed elsewhere, including but not limited to CIN, abnormal chromosomal segregation, epigenetic remodeling, centromeric function, apoptosis, and cell cycle control (75–77). Interestingly, the transcripts from human satellite II (HSATII) were linked to the RNA-driven genomic expansion, given their ability to form cDNA leading to the formation of DNA-RNA hybrids that further incorporate into pericentromeres in various human colon cancer cell lines (77). Furthermore, the presence of HSATII or GSATII (gamma satellite II) can be used as a biomarker, given that the higher ratio of HSATII to GSATII in serum is decreased in patients with cancer, contrary to the intracellular ratios (78). The discovery of novel biomarkers of CIN will allow for earlier detection and diagnosis of aneuploidy that might be caused by abnormal expression of centromeric or pericentromeric repeats.

Tumor suppressor genes such as PTEN and p53 regulate the progression of cancer, and in a recently described function, stabilize factors of centromeric and pericentromeric chromatin by regulating the transcripts emanating from these regions. For instance, PTEN interacts with pericentromeric chromatin modifier HP1α to prevent the H4K16 acetylation and stabilize the condensed chromatin state, thus repressing transcription (79). Similarly, p53-deficient mouse fibroblasts showed a 150-fold increase in transcription of SINE B1 and B2; therefore, p53 and the activation of IFN-I, as well as changes in DNA methylation, are able to regulate centromeric instability and thus lead to carcinogenesis (80). The downregulation of satncRNA by tumor suppressor genes, namely p53 and PTEN, provides a novel regulatory mechanism for the prevention and treatment of cancer but also highlights the importance of centromeric chromatin and transcript stability. In summary, transcripts that originate from these genomic loci are necessary to couple environmental signals with centromere assembly (**Figure 2**).

Environmental Signals and Centromeric Chromatin Instability

Metabolic status, macronutrients, and chromatin instability

The stability of the chromatin and the switch between euchromatin and heterochromatin readily respond to environmental cues. Epidemiologic studies have shown that nutritional status (taking into account the intake of both macro- and micronutrients) is a substantial predictor of health outcomes, and the intake of both macro- and micronutrients has been associated with CIN, either by oxidative DNA damage or dysfunctional telomeres (3, 81). In addition, Andreassi et al. (3) described the increased occurrence of micronuclei in patients with obesity, metabolic syndrome, diabetes, and cardiovascular disease, and recognized that the link between such pathologies and the generation of DNA damage requires further research. Although evidence is lacking to directly link metabolic abnormalities with chromosomal damage and malsegregation, overnutrition and physical inactivity are associated with an increased oxidative and inflammatory environment that, in turn, affects DNA function. Furthermore, several diseases that have a metabolic component, including polycystic ovary syndrome, cardiovascular disease, metabolic syndrome, and type 2 diabetes, are all strongly associated with the occurrence of micronuclei (3). In obese subjects, increased BMI was associated with a higher frequency of micronuclei, nucleoplasmic bridges, and nuclear budding, which highlights the generation of genotoxic damage in overweight and obese patients (82). Furthermore, type 2 diabetes is also associated with higher sister chromatid exchange, which enhances the rate of chromosomal aberrations (83), thus promoting abnormal cell division. Although the precise mechanisms that link metabolic disorders with chromatin instability remain poorly understood, metabolic diseases are characterized by underlying oxidative stress, which, in turn, causes damage to the DNA. For instance, in hepatocellular carcinoma, the presence of oxidative stress and inflammation leads to epigenetic instability that modifies the methylation status of DNA at different genetic loci, including tumor suppressor gene promoters, repetitive elements, and satellite DNA (84), which provides a probable link between metabolic status and the initiation of CIN. It has also been shown that under oxidative stress conditions, the addition of 1–10 μg DHA/mL to astrocyte cell culture (APOE ε3/ε3 and ε2/ε4 genotypes) was able to protect against H_2O_2 genotoxic damage by decreasing the occurrence of micronuclei (85). Moreover, protein-energy malnutrition has been found to induce chromosomal aberrations (86–88), and this phenotype was rescued by $ZnSO_4$ supplementation (87). Therefore, whether through increased inflammation and oxidative stress or via direct epigenetic landscape remodeling of crucial loci, macronutrient balance is likely perceived by the many nutrient-sensing mechanisms and can modify the balance within the cell (89), potentially leading to genomic instability (Figure 2). **Table 1** summarizes the evidence for the relations between metabolic status or macronutrient intake and micronucleus frequency or DNA damage.

Micronutrients and chromatin instability

Micronutrients act as cofactors or coadjuvators for numerous enzymatic functions, and their deficiencies have been associated with a higher rate of chromosomal abnormalities, as characterized by micronucleus formation (96). For instance, folate is a micronutrient that participates as a methyl donor in one-carbon metabolism, DNA synthesis, and cell

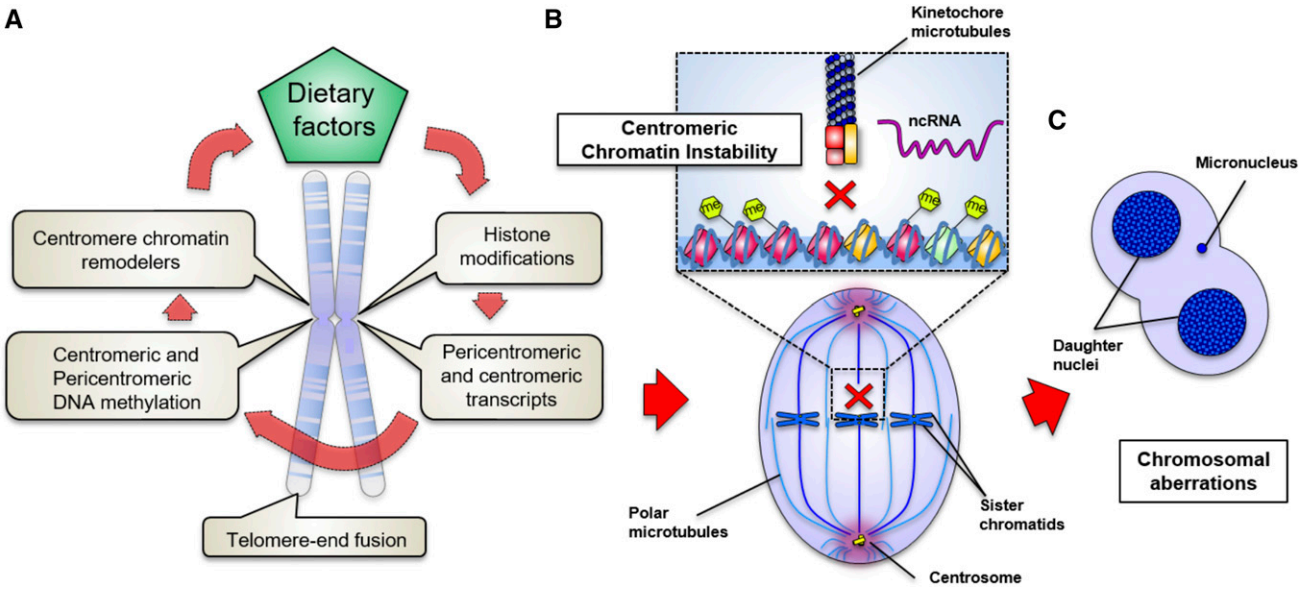

FIGURE 2 The impact of environmental factors on the stability of chromosomes. Mechanisms by which dietary and environmental factors can affect centromeric and pericentromeric chromatin stability, as well as telomeric integrity (A); cellular division is interrupted at metaphase by a deficient centromere-kinetochore interphase possibly due to altered centromeric and pericentromeric transcripts, which leads to the lagging of the chromatid (B); chromosomal aberrations that can be observed under the microscope include nucleoplasmic bridges, nuclear budding, and micronucleus formation (C). me, methyl group; ncRNA, noncoding RNA.

division. Folate deficiency, as assessed by low homocysteine concentrations, was rescued by the supplementation of folic acid, which was able to reduce the number of structural chromosomal aberrations (97, 98). Similar scenarios have also been observed for deficiencies in vitamin B-12, riboflavin, vitamin A, and minerals such as calcium and zinc (99). Conversely, supplementation with folic acid (97, 100–108), riboflavin, pantothenic acid, biotin (4), cobalamin (93, 103), vitamin C (109–115), vitamin E (114), and vitamin A and its derivatives (107, 112) was associated with decreased genotoxic damage and micronucleus reduction (4). Much research has been conducted to show the effects of folic acid supplementation and folate status on reduced micronucleus frequency. However, limited information is available on the mechanisms that underlie the prevention of these aberrations (centromeric or telomeric instability or both). Furthermore, more data are needed on whether other micronutrients may have effects on micronucleus frequency. **Table 2** summarizes studies that focus on dietary status and micronutrient supplementation and their associations with micronuclei, nucleoplasmic bridges, and DNA damage.

Environmental toxins and chromatin instability

Exposure to environmental toxicants has also been shown to induce higher gentoxic alterations. The use of common organophosphorus pesticides is linked to elevated DNA damage in human lymphocytes, and both DNA damage and micronucleus frequency in liver hepatocellular carcinoma (HepG2) cells (118). A comprehensive review on the genotoxic effect of commonly used pesticides by persons in the work place and those employed in the chemical industry was conducted by Bolognesi et al. (5), highlighting the importance of genotoxic biomonitoring in human populations who might be at a greater risk of cancer. Interestingly, many of the studies on increased micronucleus frequency in pesticide-exposed populations have established the incidence of specific polymorphisms within Paraoxonase 1 (PON1), an enzyme that detoxifies organophosphates (5, 119). This is an example of a gene-environment interaction that could help explain the modification of the relation between occupational exposures and aneuploidic events by genotype. In addition, in human colorectal carcinoma RKO cells with the functional tumor suppressor gene p53, exposure to cytotoxic agents induced both p53 and chromosomal damage (micronucleus), showing a direct relation between tumor suppressor gene expression and the induction of micronuclei (6). Therefore, the exposure to environmental insults can significantly induce CIN, which, in turn, can lead to the initiation or progression of aneuploidic events. A complete review of the causes of genomic instability was conducted previously for low-dose chemical exposure (120). Failure to repair chromosomal abnormalities in response to chronic exposures will ultimately lead to aneuploidic events, and although many environmental cues are known to affect micronucleus frequency, little is known about the regulation of centromeric and pericentromeric chromatin by environmental or nutritional signals.

Fetal programming and chromatin instability

Fetal programming of chronic adulthood diseases occurs through the transplacental communication of maternal stress, nutrition, xenobiotic exposure, etc., to the fetus, which ultimately affects the heath of the offspring. Recently it was described that the *Pon1* gene in rats is susceptible to epigenetic modifications induced by a maternal high-fat diet. Such changes to histones at the promoter region of the

TABLE 1 Studies assessing the relations between metabolic status, macronutrient intake, and chromatin instability[1]

Nutrient/nutritional status	Model	Phenotype	Year	Ref
Caloric restriction	89 monkeys ranging in age from 1 to 23 y at the time of CR (30% less than controls of same sex, age, and weight) and maintained regime for 11–15 y	CR group had a higher frequency of chromatid gaps than controls and in adult monkeys did not have an effect on aberrations related to aging	2007	(90)
Protein-energy malnutrition and infection	Peripheral blood lymphocytes of malnourished and eutrophic children with bacterial infections	Structural chromosomal aberration frequency was 5 times higher in infected malnourished children than in infected eutrophic children	2008	(86)
Protein-energy malnutrition	25 primary malnourished infants (mean age: 22 mo; range: 1–66 mo) and 25 eutrophic children from the same population by age and sex	Chromosomal aberration frequency was ~7 times higher in malnourished infants	2009	(88)
Diet rich in vegetables and plant oil rich in PUFAs	Peripheral blood lymphocytes from 76 diabetic and 21 nondiabetic patients supplemented with vegetables and plant oil daily for 8 wk	Intervention with vegetables and plant oil increased folate, γ-tocopherol, and α- and β-carotene but reduced vitamin B-12; chromosomal damage was not altered, whereas apoptosis was slightly increased	2013	(91)
Dietary methionine	Female Swiss mice fed a methionine-supplemented (2.0% dl-methionine) or methionine-deficient (0% dl-methionine) diet for 10 wk	Methionine deficiency reduced basal DNA damage in the liver, whereas methionine-supplemented diet induced DNA damage in the peripheral blood	2013	(92)
Vitamin B-12 and methionine deficiencies	Human GM13705 and GM12593 cells exposed to 13 combinations of physiologic concentrations of vitamin B-12 and methionine concentrations over 9 d	Vitamin B-12 and methionine deficiencies induce micronucleus binucleated, NBUD, NPB, and micronucleus mononucleated cells	2013	(93)
Protein-energy malnutrition	Peripheral blood lymphocytes from children with protein-energy malnutrition incubated with $ZnSO_4$	$ZnSO_4$ supplementation reduces micronuclei and NPBs and increases the nuclear division index	2014	(87)
Metabolic syndrome	Peripheral blood lymphocyte cultures of 52 MetS patients	Micronucleus frequency and comet-tail length were positively correlated with waist circumference, BMI, and plasma TGs, and negatively with HDL-cholesterol concentrations; malondialdehyde was significantly higher in MetS patients, but SOD and GPx enzyme activities were lower	2015	(94)
Vegetarian vs. nonvegetarian diet	58 healthy Swedish vegetarians and nonvegetarians aged between 21 and 37 y, analyzed for miconuclei in transferrin-positive peripheral reticulocytes	Vegetarian diet might lower genomic instability in healthy individuals; however, multivariate analysis showed no association between micronuclei and factors such as age, sex, intake of vitamins and minerals, serum folate and vitamin B-12, physical activity, or BMI	2015	(95)

[1] CR, caloric restriction; GPx, glutathione peroxidase; MetS, metabolic syndrome; NBUD, nuclear budding; NPB, nucleoplasmic bridge; Ref, reference; SOD, superoxide dismutase.

TABLE 2 Studies assessing the relations between metabolic status, micronutrient intake, and chromatin instability[1]

Nutrient/nutritional status	Model	Phenotype	Year	Ref
Niacin and chemotherapy	Male Long-Evans rats fed niacin-deficient, niacin-replete, or nicotinic acid–supplemented diets for 3 wk and gavaged with etoposide	Niacin deficiency increased spontaneous micronucleus and SCE frequency; etoposide treatment increased micronuclei and SCEs, but the absolute increases were greater with niacin deficiency	2002	(116)
Folic acid	Human lymphocytes from female patients cultured with low or high doses of folic acid and methyltetrahydrofolate	Low folic acid caused aneuploidy of chromosomes 17 and 21 associated with breast cancer and leukemia	2004	(100)
MTHFR polymorphism C677T (rs1801133) and folic acid	Human lymphocytes of 12 subjects with 4 MTHFR genotypes	Increased micronuclei with low folic acid with MTHFR allele (both homozygotes and heterozygotes); genotype was not significant; folic acid deprivation was associated with global DNA hypermethylation	2006	(101)
Folic acid or 5-methyltetrahydrofolate	Human lymphocytes incubated for 9 d	Folic acid deprivation especially increased NBUD- and micronucleus-containing telomeric DNA, NBUDs with interstitial DNA, and NBUDs and micronuclei with both centromeric and telomeric DNA; whole chromosomes were rare but also appeared to exist in NBUDs	2007	(97)
Folic acid	Randomized clinical trial for aspirin and folic acid prevention of colorectal adenomas; colon biopsies of normal-appearing mucosa from the right and left	No associations between L1 methylation and folate status, physiologic variables, or circulating B vitamins, homocysteine, or selected genotypes; however, race, folate, and plasma vitamin B-6 showed associations with global methylation difference between the right and the left bowels	2009	(102)
Folic acid and vitamin B-12	Human lymphocytes from 160 patients with history of cancer	12 patients with increased mean micronucleus frequencies, from which 10 patients were reduced by 33.5% after the intervention with the 2 micronutrients	2012	(103)
T2D and folic acid	30 patients with T2D prescribed oral doses of folic acid for 1 mo and 30 controls	Free radicals induced DNA damage in T2D, but folic acid reduced 8-OHdG, lipid peroxides and micronuclei	2012	(104)
Choline, folic acid, or both	Human lymphocytes from 6 patients cultured in 18 combinations of choline and folic acid media for 9 d	Frequencies of micronuclei, NBUDs, and necrosis were inversely correlated with choline maintaining folic acid constant; folate effects were 2.5- to 6.2-fold greater	2012	(105)
CVD and dietary status and vitamin C	34 children and adolescents with different levels of CVD risk	Vitamin C intake was inversely correlated with DNA damage, and micronucleus frequency was inversely correlated with folate	2012	(109)
Vitamin B-12 and methionine deficiencies	Human GM13705 and GM12593 cells exposed to 13 combinations of physiologic concentrations of vitamin B-12 and methionine concentrations over 9 d	Vitamin B-12 and methionine deficiencies induced micronucleus binucleated, NBUD, NPB, and micronucleus mononucleated cells	2013	(93)
Vitamins A and C	29 students before and after recommended daily dose of vitamin for 30 d	Micronuclei in cells and total level of cytogenetic disorders decreased 38% after vitamin course, with a slight increase in the percentage of apoptotic cells	2013	(110)
Vitamin C plus dietary polyphenols ferulic acid, gallic acid, chlorogenic acid, or epigallocatechin gallate	Male Swiss albino mice orally administered methylurea plus sodium nitrite and analyzed bone marrow cells	Ferulic acid, gallic acid, chlorogenic acid, or epigallocatechin gallate had protective effects; combinations of vitamin C plus polyphenols showed a further protective effect; reduction in micronuclei by coadministration of a combination of polyphenols with methylurea plus sodium nitrite, and a similar trend for metaphase chromosome aberrations	2013	(111)

(Continued)

TABLE 2 (*Continued*)

Nutrient/nutritional status	Model	Phenotype	Year	Ref
Vitamin complex containing vitamin C, α-tocopherol, and β-carotene	Peripheral blood mononuclear cells of healthy individuals aged 40–85 y	Vitamin complex induced micronuclei; no correlation between ROS and micronuclei in the presence or in the absence of the vitamin complex	2013	(112)
Vitamin C	Buccal cells from 108 healthy females supplemented with amfepramone to induced DNA damage	Vitamin C supplementation decreased micronuclei and apoptosis	2013	(113)
Vitamins A, C, and E and selenium	Erythrocytes of Nile tilapia (*Oreochromis niloticus*) exposed for 5 and 7 d to sublethal concentrations of combined metals including cadmium, copper, lead, and zinc	Vitamin E addition to the diet decreased micronuclei, micronucleus binucleated cells, and most nuclear abnormalities and morphologic alterations than in metal-treated groups; combination of selenium with vitamins A, C, and E had superior effect	2014	(114)
Vitamin D	208 male and female participants exposed to solar UV light with different serum vitamin D concentrations	Methylation in L1 decreased with increasing solar UV exposure; no correlation between L1 methylation and micronuclei but increase in NPBs and necrosis; no effect of vitamin D	2014	(117)
Folic acid	Human WIL2-NS cells with 30, 300, or 3000 nmol folic acid/L for 42 d	Global DNA hypomethylation with folic deficiency; negative association between folic acid and uracil incorporation into telomeric DNA; deficiency resulted in 60% of micronucleus-containing acentric terminal fragments, consistent with 3-fold increase in terminal deletions	2014	(106)
Micronutrients and SNPs for MTHFR C677T (rs1801133), MTHFR A1298C (rs1801131), MTR A2756G (rs1805087), MTRR A66G (rs1801394), XRCC1 Arg399Gln G28152A (rs25487), RFC G80A (rs1051266), GST Mu, GST Theta, and XRCC3 Thr241Met C18067T (rs861539)	Cross-sectional study in 462 healthy children aged 3, 6, and 9 y	Ca^{2+} positively associated with micronuclei and necrosis; α-tocopherol negatively associated with apoptosis, nuclear division index, and NPBs, but positively associated with necrosis; lutein positively associated with NPBs	2015	(107)
Folic acid	Male Balb/c mouse reticulocytes and normochromatic erythrocytes	Genotoxic effect of folate deficiency, with modest mutagenic effect on Pig-a locus, and comparable micronucleus formation to known mutagens	2015	(108)
Vitamin C	Erythrocytes from pregnant hairless rat dams exposed to UV-A light for 6 d during gestation and their fetuses	Micronuclei and micronucleus polychromatic erythrocytes observed in neonates born to mothers exposed to UV-A for 40, 80, or 160 min; vitamin C reduced both markers of damage	2015	(115)

[1] CVD, cardiovascular disease; GST, glutathione S-transferase; L1, long interspersed nuclear element 1; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; NBUD, nuclear budding; NPB, nucleoplasmic bridge; Pig-a, phosphatidylinositol glycan anchor biosynthesis class A; Ref, reference; RFC, reduced folate carrier; ROS, reactive oxygen species; SCE, sister chromatid exchange; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells 1; XRCC3, X-ray repair complementing defective repair in Chinese hamster cells 3; 8-OHdG, 8-oxo-2′-deoxyguanosine.

gene might have sex-specific consequences for the function of the antioxidant defense system in the offspring (121). Maternal caloric overnutrition has the capacity to modify the epigenetic landscape of several antioxidant mechanisms in the offspring (122), as well as impair the inflammatory response (123), thus contributing to disease susceptibility of the offspring later in life. Maternal exposure to environmental toxicants can also affect fetal development. A study by Levario-Carrillo et al. (124) used a cytokinesis block micronucleus cytome to assess the frequency of micronuclei in cells from cord blood and found that the frequency at which aneuploidic events occur was intimately related to the maternal geographic environment (urban or rural) as well as to pregnancy outcomes. Furthermore, there was a positive correlation between micronucleus frequency between mothers and their newborns. Although additional research is greatly needed, these data suggest that the maternal environment has the ability to both alter maternal physiology and shape the offspring's susceptibility to CIN. The regulation of chromosomal stability depends entirely on the antioxidant systems and the repair mechanisms that monitor the progression and fidelity of the genetic material during cell division.

The direct mechanisms linking the fetal environment, chromosomal abnormalities, and adulthood diseases remain poorly understood and require further research. It is possible that the initial genotoxic insults in utero (in response to diet or other maternal environmental factors) could affect not only the expression of the genes within each chromosome but the structural properties of the chromosome itself, either by a genetic mutation or epigenetic modification. Nucleoplasmic bridges, telomere-end fusions, nuclear budding, and other chromosomal abnormalities were found to be good predictors of several carcinomas or the advancement of chronic metabolic illnesses. The methylation pattern of satellite DNA from centromeres and pericentromeric regions is consistently present in most carcinogenic processes (125–127) in response to stress signals (128, 129), which can be mediated by the overexpression of DNA methylases (130, 131). Similarly, abnormal overgrowth and differentiation of the placenta result in the hypomethylation of L1 repetitive element (132). The abnormal hypo- or hypermethylated state thus translates into the inability of chromosomes to segregate appropriately during mitosis (133). Repetitive elements, such as satellite DNA or pericentric transposable elements, are ultimately the targets that are more susceptible to epigenetic regulation by diet (134).

Conclusions

Cancer initiation and progression have been the focus of intensive research for many years. The lifestyle factors that underlie the generation of cancer likely act by causing systemic inflammation, oxidative stress, cell cycle deregulation, and DNA damage, among numerous others (81). This multitude of mechanisms only explains in part the forces that drive aneuploidic events, but the micronucleus assay has become indispensable for accurately assessing the earliest cellular events that may drive aneuploidy in response to genotoxic stimuli. Many environmental and dietary factors have been proposed to cause the initiation of DNA damage, centromeric and telomeric instability, and telomeric fusion (5–7). Transcriptional control of centromeric and pericentric transcripts that emanate from previously thought silent heterochromatic regions expands our understanding of how the centromere is stabilized; whether and how diet and the environment may prime the chromatin at these regions has not been assessed.

Current research has focused on defining the mechanisms that contribute to the establishment and preservation of the chromatin within centromeres; however, only limited research has explored the effects of environmental factors that can promote either stability or imbalance of the centromeres (3, 8, 81). Studies that address the direct link between nutritional excess or deficiency and the methylation status of the centromere will help expand the relation between nutritional and environmental cues and the plasticity of this epigenetic mark within this chromosomal region. Moreover, the effect that such methylation modifications have on the establishment of centromeric proteins and the kinetochore machinery will provide more evidence on the influence of nutritional adequacy on cell division and viability. Finally, given the recent discovery of centromeric and pericentromeric transcripts and their role in the complex centromere chromatin assembly (76, 77), future studies should assess the transcriptional regulation of such regions in response to different environmental stimuli. Given the inflammatory and oxidative nature of metabolic diseases (e.g., obesity, diabetes, metabolic syndrome, and cardiovascular disease), more research is also needed to determine whether metabolic abnormalities are accompanied by the generation of DNA damage within the centromeres or telomeres, leading to chromosomal missegregation.

There is mounting evidence from epidemiologic, environmental, and nutritional studies that lifestyle and dietary factors affect the origination of aneuploidic events. Nevertheless, more studies in humans, animals, and cell models are needed to understand the epigenetic regulation of the centromere in response to nutritional or environmental stress, and how this accounts for the initiation of aneuploidy.

Acknowledgments

We thank Brett R Loman and Tzu-wen Liu from the Division of Nutritional Sciences at University of Illinois at Urbana-Champaign for their constructive comments and advice during the preparation of the manuscript. The authors' responsibilities were as follows—Y-XP, PO-W, and DH-S: were responsible for the conception of the manuscript; DH-S and RSS: drafted and finalized the manuscript; Y-XP and DH-S: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

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